

PRODUCT INFORMATION

Neospora caninum SOLUBLE PROTEIN EXTRACT

PRODUCT DESCRIPTION

Soluble protein extract is obtained from *Neospora caninum* tachyzoites growth in cell cultures of the MARC-145 cell line. Soluble extract is produced from tachyzoites purified by gel filtration that removes essentially all MARC-145 cell proteins and disrupted by ultrasound treatment for obtaining soluble proteins. The soluble extract has been optimized and is batch-to-batch tested to be used as antigen in ELISA for serological diagnosis of neosporosis in cattle and other ruminants^{1,2}.

REAGENTS

The soluble extract is provided as a solution in 0.01 M Tris-ClH, pH 7, containing 2 mM of protease inhibitor PMSF as a preservative.

PRODUCT PROFILE

Protein profile of each batch is validated according to Bradford concentration by Coomassie SDS-PAGE by comparison with a reference soluble extract. We include specific information about the protein concentration for each batch together with the protein quantity required per well of the ELISA plate for the detection of specific antibodies against *N. caninum* in bovine sera in the quality product document. Required protein quantity of soluble extract per well is defined as the quantity of soluble extract required to give an absorbance of 1.1 in the positive controlwell at 405 nm after 15-30 minutes. Basically microtiter plates are coated with soluble extract in 0.05 M carbonate/bicarbonate buffer, pH 9.6. An anti-IgG1/IgG2 bovine antibody conjugated with peroxidase and ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) as colorimetric substrate at 25 °C are used. Negative and positive reference bovine sera from experimentally and naturally infected animals with *N. caninum* are employed and the ratio obtained between optical density achieved by positive and negative control sera is ≥ 8 - 12<.

Facultad de Veterinaria
Pabellón Central. Planta -3
Universidad Complutense de Madrid
Avenida Puerta de Hierro s/n
28040 Madrid, España
Tlf. 629 50 82 83
Fax. 616 56 32 98
salvet.innova@gmail.com
www.salvetinnova.com



STORAGE

For extended storage, the solution should be frozen at $-80\text{ }^{\circ}\text{C}$ working in aliquots. Repeated freezing and thawing is not recommended. PMSF is an inhibitor of proteases used as preservative. PMSF is very unstable in the presence of water. The half-life of aqueous PMSF at $25\text{ }^{\circ}\text{C}$ at pH 7.0 is 110 minutes². We recommend the use of the soluble extract for a period not longer than one year after the first thawing to avoid protein degradation. If turbidity or precipitation occurs upon prolonged storage, discard its use.

References

1. Alvarez-García G, García-Culebras A, Gutiérrez-Expósito D, Navarro-Lozano V, Pastor-Fernández I, Ortega-Mora LM. *Serological diagnosis of bovine neosporosis: A comparative study of commercially available ELISA tests. Veterinary Parasitology. Volume 198, Issues 1–2, 15 November 2013, Pages 85-95.*
2. Alvarez-García G, Collantes-Fernández E, Costas E, Rebordosa X, Ortega-Mora LM. Influence of age and purpose for testing on the cut-off selection of serological methods in bovine neosporosis. *Vet Res.* 2003 May-Jun; 34(3):341-52.
3. Gordon T. James. *Inactivation of the protease inhibitor phenylmethylsulfonyl fluoride in buffers. Analytical Biochemistry. Volume 86, Issue 2, 1 June 1978, Pages 574-579.*



PRODUCT INFORMATION

Neospora caninum FORMALDEHYDE-FIXED TACHYZOITES

PRODUCT DESCRIPTION

Neospora caninum tachyzoites are grown in cell cultures of the MARC-145 cell line. Tachyzoites are purified by gel filtration that removes essentially all MARC-145 cell debris and fixed in buffered 0.2% formaldehyde.

Formaldehyde-fixed *N. caninum* tachyzoites production has been optimized and is batch-to-batch tested to be used as antigen in IFAT for serological diagnosis.

REAGENTS

Fixed *N. caninum* tachyzoites are provided as a suspension in phosphate buffered saline, pH 7.4, containing 0.2 % formaldehyde*. *N. caninum* isolate is the Nc1.

PRODUCT PROFILE

Each vial contains 10^7 formaldehyde-fixed tachyzoites. We recommend homogenizing the tachyzoite suspension by passing through 25G needle prior to each use. The recommended number of tachyzoites per 4 mm-diameter well is 10^5 tachyzoites (10 μ l of tachyzoite suspension/ well) for IFAT analysis.

STORAGE

Keep the vials refrigerated at a temperature between 2 and 8 ° C up to one year. Do not freeze.

* This product contains formaldehyde. Use appropriate equipment and avoid inhalation and contact with skin and eyes. Consult formaldehyde hazard and precautionary statements before use.



PRODUCT INFORMATION

***Neospora caninum* TACHYZOITES**

PRODUCT DESCRIPTION

Neospora caninum tachyzoites are obtained from cell cultures of the MARC-145 cell line. Tachyzoites are purified by gel filtration that removes essentially all MARC-145 cell debris. Tachyzoites are washed with PBS containing 2 mM of protease inhibitor PMSF* as a preservative and pelleted by centrifugation.

REAGENTS

Neospora caninum tachyzoites are provided as a pellet. *Neospora caninum* isolate is Nc-1.

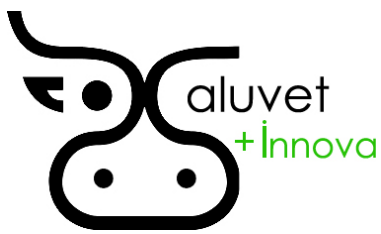
PRODUCT PROFILE

N. caninum tachyzoites can be used for the extraction of nucleic acids or proteins from the parasite. Tachyzoites can be also used as *N. caninum* antigen for Western blotting by resuspending the tachyzoite pellet in lysis buffer or directly in acrylamide gel's SDS-loading buffer and heating > 90°C for 2 minutes. We include specific information about the amount of tachyzoites per pellet in the product package label .

STORAGE

The pellet should be frozen at – 80 °C up to one year. Repeated freezing and thawing is not recommended.

* This product contains PMSF. Use appropriate equipment and avoid inhalation. Consult PMSF hazard and precautionary statements before use.



PRODUCT INFORMATION

***Neospora caninum* DNA**

PRODUCT DESCRIPTION

Genomic DNA from *Neospora caninum* is obtained from purified tachyzoites growth in cell cultures of the MARC-145 cell line. DNA extraction is carried out by conventional methods removing RNA by RNase treatment.

REAGENTS

Neospora caninum DNA is provided in a double distilled and deionized water solution (molecular biology quality).

PRODUCT PROFILE

Neospora caninum DNA can be used as template and positive control for *N. caninum* PCR and quantitative PCR. We include specific information about the concentration and purity of DNA determined by spectrophotometry at 280/260 nm in the product package label. The equivalence of DNA concentration present in a 1 microliter of DNA solution with the number of tachyzoites is also given.

STORAGE

DNA solution should be frozen at -20 °C up to one year. For extended storage, the solution should be frozen at - 80 °C (specially for quantitative PCR use). Repeated freezing and thawing is not recommended. Prepare aliquots of DNA solution previous to storage.